# Chemotherapy induced intestinal mucosal barrier damage: a cause of falsely elevated serum 1,3-beta-d-glucan levels?

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Running Head: Serum BDG levels in intestinal mucositis

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Abstract

Blood citrulline and intestinal fatty acid binding protein were determined as biomarkers for intestinal mucositis. Biomarker levels were correlated with corresponding serum 1,3-beta-d-glucan levels in 56 samples obtained from 33 cases with underlying hematological malignancies receiving induction chemotherapy. No correlation between biomarkers of intestinal mucositis and BDG levels was observed.

(1→3)-β-D-Glucan (BDG) is a polysaccharide cell wall component of most fungal species and a useful serum biomarker for early diagnosis of invasive fungal infections (IFIs) (1, 2). False positive results have been linked to use of cellulose containing haemodialysis membranes, blood-derivates, broad spectrum antibiotics, and severe mucositis (3). Intestinal mucositis is a frequently observed adverse event of induction chemotherapy in patients with underlying hematological malignancies (4). Severe mucositis is characterized by a loss of integrity of the intestinal mucosal barrier, increasing the likelihood of translocation of bacterial and/or fungal commensals of the gastrointestinal tract (5). Whether intestinal mucositis is a cause of false positive BDG serum levels has been insufficiently evaluated to date mainly because of difficulties in diagnosing and defining mucositis in hematological malignancy patients in clinical routine. Biopsy of the gastrointestinal tract is the diagnostic gold-standard, but often hindered by the unfavorable risk-benefit ratio in those with severe underlying conditions and increased risk of bleeding.

Thus, several clinical scores have been implemented for diagnosis and grading of mucositis (6, 7), but these are prone to subjective interpretation. Therefore, noninvasive diagnostic biomarkers for mucositis have been increasingly investigated. Serum citrulline, a non-protein amino acid produced by intact enterocytes (8, 9), and intestinal fatty acid binding protein (IFABP), low-molecular mass cytosolic proteins found in tissues involved in the uptake, intracellular metabolism and transport of long-chain fatty acids (10, 11), are two of the most promising biomarkers for detection of loss of enterocytes and therefore mucosal barrier injury.

The objective of this study was to investigate whether serum BDG levels are falsely elevated in hematological malignancy patients with intestinal mucositis. A total of 56 same day plasma and serum samples, were prospectively obtained from 33 consecutive cases (i.e. 33 admissions corresponding to 24 patients; maximum 2 samples per case) with underlying



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hematological malignancies receiving induction chemotherapy for acute leukemia. None of our patients fulfilled criteria for IFI according to 2008 revised definitions for IFIs from the EORTC/MSG group (12, 13). Samples were collected between July 2012 and May 2013 at the University Hospital of Graz, Austria, and a maximum of two samples per case were included (in those with two samples, samples were obtained always > 4 days apart). BDG was determined prospectively, while citrulline and IFABP were determined retrospectively in serum/plasma samples after immediate sample storage at -70° at the Medical University of Graz, Austria. BDG was determined using the automated protocol Fungitell® assay (Associates of Cape Cod, Inc., East Falmouth, USA), as described previously (14). IFABP was determined using the Quantikine Human FABP2/I-FABP Immunoassay (R&D Systems Europe Ltd., OX, UK) according to the manufacturer's instruction. Citrulline was determined from plasma samples at the University Hospital Leuven, Belgium, using liquid chromatograph-tandem mass spectrometry as described elsewhere (15). Mucositis was classified according to citrulline levels: >30µmol/L as no evidence of mucositis, 20-30µmol/L as partial villus atrophy, 10-20µmol/L as destructive mucosal lesions and <10µmol/L as diffuse destructive mucositis (16, 17). IFABP was used as a second marker for mucositis to better support mucositis classification based on citrulline levels. In contrast to citrulline IFABP levels are highly elevated in case of intestinal mucosal barrier damage, with 100 fold increases in patients with intestinal ischemia when compared to healthy controls (10).

For statistical analyses a cut-off of 10µmol/L citrulline was used as indicator for extensive mucositis. The study adhered to the Declaration of Helsinki (1996) and Good Clinical Practice. The study protocol was approved by the local ethics committee, Medical University Graz, Austria (EC-number 23-343) and registered at ClinicalTrials.gov (identifier NCT01576653). Informed consent was obtained from all participating patients. For statistical analysis SPSS Version 23 was used (SPSS Inc., Chicago, IL, USA). For continuous variables



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medians plus interquartile range (IQR) or mean plus 95% confidence interval (95% CI) are displayed as appropriate. Correlation analysis was performed by using Spearman-Rho analyses due to non-normality of our data and data are presented in scatterplots. Retrospective power analysis revealed that a sample size of 48 mucositis samples (8 samples were derived from patients without mucositis) provides at least 80% power (with alpha = 0.05) to detect a correlation of r=0.44 (i.e. $r^2$=0.19) or higher for correlations of three variables. Chi-squared test was used for categorical data. A p-value of <0.05 was considered statistically significant.

Demographic data, underlying diseases, and biomarker levels for patients, cases and samples are displayed in Table1. All patients were receiving mold-active antifungal prophylaxis with posaconazole oral suspension, as recommended in clinical guidelines. Additionally, all included patients were able to tolerate solid food. All but two patients were on systemic antibiotic treatment/prophylaxis at the time of sample collection. Most common antibiotics prescribed at the time of sampling were: levofloxacin (23 samples, 42.6%), cefepime (21 samples, 38.9%), linezolid (7 samples, 13%) and meropenem (6 samples, 11.1%). Additionally, all patient received prophylaxis for oral mucositis containing chlorhexidine mouth wash and nystatin oral solution. Overall specificity of serum BDG testing in our cohort was 0.95 (95% CI 0.85 – 0.98).

Positive BDG serum levels (>80pg/ml) were observed in three serum samples: two out of 17 samples (11.8%) with citrulline levels <10µmol/L had false positive BDG levels (120.5pg/ml and 149.3pg/ml respectively; corresponding IFABP levels: 1366.3pg/ml and 976.1pg/ml respectivley) and one out of 39 samples (2.6%) with citrulline levels ≥10µmol/L had a false positive BDG level (232.4pg/ml; corresponding IFABP level: 380.1pg/ml; p=0.160). Median BDG levels in samples with citrulline levels <10µmol/L and ≥10µmol/L were 15.4pg/ml in both groups. Serum IFABP levels showed a strong negative correlation with plasma citrulline levels (Spearman-Rho r=-0.601; p=<0.001). Additionally, IFABP values were significantly

higher in the mucositis group (i.e. n=17 with <10µmol/L citrulline) compared to the group without severe mucositis according to citrulline levels (814.8pg/ml versus 380.9pg/ml; p=0.004) supporting mucositis diagnosis based on citrulline levels. Correlations between BDG and citrulline, or IFABP are displayed in Figure 1.

Our main objective in this study was to determine whether underlying intestinal mucositis in hematological malignancy patients is a potential cause of falsely elevated serum BDG levels. As surrogate markers for mucositis we used citrulline and IFABP. Both markers showed excellent correlation with each other supporting the diagnosis of intestinal mucositis, however, no correlation between either citrulline or IFABP and serum BDG levels was observed. Additionally, only 2 out of 17 samples from patients with biomarker levels suggestive for diffuse intestinal mucosal lesion yielded positive serum BDG levels.

As fungi, especially *Candida spp*., are commensals in the gastrointestinal tract, mucosal barrier damage is considered a risk factor for developing IFIs (18, 19). Antifungal prophylaxis is therefore a recommended practice in patients with hematological malignancies receiving induction chemotherapy or hematopoietic stem cell transplantation (20). In our setting oral posaconazole suspension was used as prophylactic agent in all patients. Antifungal prophylaxis is likely to result in a decreased fungal burden in the gastrointestinal tract and therefore a reduced amount of fungal components in serum in case of mucosal barrier damage. This may be particularly true for the posaconazole suspension that was used in all our patients. Due to the liquid formulation, posaconazole suspension is acting more fungicidal in the upper gastrointestinal tract (i.e. oral, esophagus) when compared to the newer tablet formulation. As fungal colonization is mainly found in the upper gastrointestinal tract, posaconazole suspension may cause more substantial reduction of fungal colonization when compared to the tablet or the intravenous form. While the fact that antifungal prophylaxis was used in all participants may be considered a limitation from a scientific standpoint (i.e. we



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could not evaluate whether mucositis is associated with false positive BDG levels in the absence of antifungal prophylaxis), it may be considered as strength from a clinical standpoint as this reflects clinical practice in most specialized centers.

Specificity of serum BDG testing in our study was 95% which is comparable to other studies in hematological malignancy patients (21). Our results therefore clearly indicate that mucositis may not be a major cause of false positive BDG levels in patients with induction chemotherapy, receiving antifungal prophylaxis. These findings have important clinical implications, as false negative BDG test results in the presence of antifungal therapy or prophylaxis have been shown to occur rarely. This is supported by a study conducted by Koo et al., who could demonstrate that systemic antifungal therapy did not alter the performance of serum BDG determination (22).

Our study was limited by indirect assessment of intestinal mucositis by citrulline and IFABP determination. No endoscopies to verify intestinal mucosal barrier damage were performed. Additionally, citrulline and IFABP are both produced in the lower gastrointestinal tract, and may not be very reliable biomarkers in patients with exclusively oral mucositis. Oral mucositis may have therefore been underestimated in our study. Small samples size was another important limitation.

In conclusion, we did not observe elevated serum BDG levels in hematological malignancy patients with underlying intestinal mucositis who received antifungal prophylaxis, indicating that serum BDG may be a specific biomarker in this setting.



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**Conflicts of interest**

Reinhard B. Raggam received travel grants from Pfizer and Merck.

A.Wölfler received research grants and speaker honoraria from Merck.

M. Hoenigl received research grants from Merck and Pfizer; served on the speakers' bureau of Pfizer, Gilead, Astellas, Basilea and Merck and received travel grants from Astellas, Merck, Gilead and Pfizer.

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**Figure 1 Correlation of 1,3-beta-d-glucan (BDG) with citrulline and intestinal fatty acid binding protein (IFABP) in same day plasma and serum samples.** Correlation analyses were performed by Spearman-Rho-analysis and yielded no significant correlations between BDG and citrulline (r=0.081) and BDG and IFABP (r=0.048).

**Table 1** Demographic data, underlying diseases and 1,3-beta-d-glucan (BDG), citrulline and intestinal fatty acid binding protein (IFABP) levels in the study population.

| | **Patients* [n (%)]** | **Cases** [n (%)]** | **Samples*** [n (%)]** |
|---|---|---|---|
| N | 24 | 33 | 56 |
| Sex: Female / Male | 10 (41.7) / 14 (58.3) | 15 (45.5) / 18 (54.5) | 28 (50) / 28 (50) |
| Age, years (range) | 54 (20-67) | 57 (20-67) | 59 (20-67) |
| Underlying diseases | | | |
| Acute myeloid leukemia | 18 (75) | 26 (78.8) | 46 (82.1) |
| Acute lymphoblastic leukemia | 3 (12.5) | 4 (12.1) | 5 (8.9) |
| Myelodysplastic syndrome | 2 (8.3) | 2 (6.1) | 3 (5.4) |
| Aplastic anemia | 1 (4.2) | 1 (3) | 2 (3.6) |
| Citrulline µmol/L, median (IQR) | 14.3 (8.6-33.6) | 14.1 (8.8-18.2) | 13.8 (8.7-18.2) |
| Citrulline <10µmol/L (diffuse destructive mucositis) | 8 (33.3) | 11 (33.3) | 17 (30.4) |
| Citrulline 10-20µmol/L (destructive mucosal lesions) | 12 (50) | 18 (54.5) | 31 (55.4) |
| Citrulline 20-30µmol/L (partial villus atrophy) | 1 (4.2) | 1 (3) | 4 (7.1) |
| Citrulline ≥30µmol/L (no evidence of mucositis) | 3 (12.5) | 3 (9.1) | 4 (7.1) |
| BDG pg/ml, median (IQR) | 15.4 (7.4-39.9) | 15.4 (5-35.8) | 15.4 (6.8-30.9) |
| BDG ≥80pg/ml | 2 (8.3) | 2 (6.1) | 3 (5.4) |
| BDG <80pg/ml | 22 (91.7) | 31 (93.9) | 53 (94.6) |
| IFABP pg/ml, median (IQR) | 524.6 (309.8-804.3) | 483.5 (332.3-849.7) | 514.9 (337.9-792.6) |
| Severe Diarrhea | 3 (12.5) | 3 (9.1) | 4 (7.1) |
| Citrulline <10µmol/L (diffuse | 1 (33.3) | 1 (33.3) | 2 (50) |

| | | | |
|---|---|---|---|
| destructive mucositis)<br>Citrulline 10-20µmol/L (destructive mucosal lesions) | 2 (66.6) | 2 (66.6) | 2 (50) |

Absolute numbers plus percentage displayed, unless otherwise noted.
*Patients = first sample per patient within the study period; **Cases = 33 admissions corresponding to 24 patients; ***Samples = all samples obtained from included patients within the study period
Abbreviations: BDG = 1,3-beta-d-glucan; IQR = 25 -75 interquartile range; IFABP = intestinal fatty acid binding protein